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Studies on Bacteriophage Distribution: Virulent and Temperate Bacteriophage Content of Mammalian Feces

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Freshly voided samples of the feces of cows, pigs, and humans were analyzed for the enumeration of cell-free plaque-forming units (PFU) of coliphages and *Salmonella* phages. Coliphage PFU counts per gram (wet weight) of feces were found to range from less than 10^1 to $>10^7$. *Salmonella* phages were found in three out of five porcine samples, but none were found in the four bovine samples analyzed. Virulent coliphages related to the ϕX174/S13 serological group showed some "habitat preference" in that the S13 type of phages was found only in pig feces, whereas the ϕX174 type of phages was found only in cow dung. Temperate coliphages were detectable in a majority of samples of both human and porcine origin but were infrequently found in bovine samples. About 80% of the temperate coliphages of fecal origin have been found to be serologically related to phage HK022 (Dhillon and Dhillon, 1973), and all are efficiently inducible by ultraviolet light irradiation. However, considerable diversity within the group was found when the prophage immunity pattern of 10 randomly selected isolates was examined.

With a view to understanding the ecological aspects of bacteriophages, we have previously presented data on total coliphage contents of sewage (5, 8) and data on the density of ribonucleic acid (RNA)-containing, male-specific coliphages in sewage (4). One conspicuous feature of our published and unpublished experimental observations is the rarity of temperate phages in sewage, which is in marked contrast to the very high density of diverse types of virulent phages (5, 7, 8). Efficient isolation of temperate coliphages has been accomplished in the prophage form by screening for lysogenic bacteria (1, 10, 11; our unpublished data). From this it became obvious that an even more efficient isolation of temperate coliphages should be possible by dispensing with the step of bacterial isolation and plating the *Escherichia coli*-rich mammalian fecal samples directly onto the indicator cells. By the adoption of this simple modification we have succeeded in isolating large numbers of temperate coliphages from mammalian feces. Our findings on the total coliphage content and the incidence of temperate coliphages in the feces of three mammalian species are recorded in this communication.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The bacterial and bacteriophage strains are described in Table 1.

Media. Tryptone broth agar (TBA) was used for plaque-forming unit (PFU) assays. Colony-forming unit (CFU) assays were made on nutrient agar (NA). Dehydrated tryptone and NA were purchased from Difco Manufacturing Co. (Detroit, Mich.). Phage dilutions were made either in tryptone broth (TB) or in phosphate saline (0.02 M sodium phosphate, 0.5% sodium chloride, pH 7.0 [P-Sal]), or in the diluent prepared by adding 0.1% (wt/vol) of tryptone granules to P-Sal. Basic phage methods and composition of P-Sal, TB, TBA, and NA have been published elsewhere (2, 3). Special methods pertinent to this study are described below.

Sample sources. For cows and pigs, samples were collected from farm animals reared under normal farm conditions in Hong Kong. To obtain the greatest possible diversity, only one sample was obtained from a single farm. Human samples were taken from university students coming from different families. The numbers of samples analyzed were the following: cows, 13 samples; pigs, 21 samples; and humans, 22 samples.

CFU and PFU assays. Only freshly defecated samples were used, and these were collected in sterile test tubes of known weight. After determining the wet weight of a sample, P-Sal was added to make 0.1 g of fecal matter per ml of suspension. Samples of these suspensions were plated on NA plates for total CFU counts. The resulting colonies were not subjected to any diagnostic tests, and no attempts were made to determine the numbers of bacteria belonging to different genera or even higher taxa. Remaining suspensions were centrifuged at 5,000 rpm for 30 min. Two to four milliliters of superna-

TABLE 1. *Bacterial and bacteriophage strains*

Strain no.	Properties[a]	Reference/origin/source
Bacterial strains		
Escherichia coli:		
TD6	High-frequency recombination male, HfrH	Hayes (9)
TD9	K-12, F^-	Dhillon and Dhillon (7)
TD57	*E. coli* C, strain Cla, F^-	Sasaki and Bertani (13)
TD230	Cla derivative resistant to S13, ϕX174, and HK047	Dhillon and Dhillon (7)
TD244	Female (F^-) derivative of TD6	Selected for this study
TD248	HK219-resistant derivative of TD244	Selected for this study
TD252	HK221-resistant derivative of TD244	Selected for this study
TD257	Phage T1-resistant (*tonA*) derivative of TD57; also resistant to phage HK022	Selected for this study
TD262	TD9 derivative sensitive to phage S13 but resistant to ϕX174; selected for resistance to phage HK019	Dhillon and Dhillon (7)
TD298	F^- *lac*$^-$	Dhillon and Dhillon (4)
TD299	Lac$^-$/F′ *lac*$^+$	Dhillon and Dhillon (4)
Salmonella typhimurium:		
TD28	Strain LT2	Zinder and Lederberg (15)
Bacteriophage strains		
HK019	Virulent phage	Dhillon and Dhillon (7)
HK022	Temperate phage; does not adsorb to *tonA* mutants of *E. coli*	Dhillon and Dhillon (7)
HK047	Virulent phage related to S13 and ϕX174	Dhillon and Dhillon (7)
HK219	Virulent phage, host range M	Dhillon and Dhillon (unpublished data)
HK221	Virulent phage, host range M	Dhillon and Dhillon (unpublished data)
HK239	Temperate phage related to P2	Dhillon and Dhillon (2)
T1	Virulent phage	A. D. Hershey
S13	Virulent phage	I. Tessman
ϕX174	Virulent phage	R. Sinsheimer (14)

[a] Genetic symbols: F^-, absence of F-factor and female sex; F′, presence of modified (F-prime) form of F-factor; *lac*$^+$ and *lac*$^-$, ability and inability to ferment lactose, respectively.

tant fluid was agitated with approximately 0.25 ml of chloroform and stored at 5°C. After chloroform had settled, portions of each sample (a total of 56 samples) were plated on strain TD57 for PFU assay by the agar overlay technique. Samples that gave no plaques were replated with 0.4-ml portions of undiluted suspensions, using a minimum of three plates. If no plaques were seen, the sample was classed as "negative" (see Table 3). Plaques resulting from "positive" samples were counted at the appropriate dilutions, and PFU estimates per gram were made (Table 2). Methods for preparation of indicator cultures have been described previously (5).

Out of a total of 56 samples, 9 samples were randomly selected. Portions of these were plated on strain TD28 for the detection of phages able to form plaques on strain TD28 of *Salmonella typhimurium*. These nine samples were also used to select and characterize phage isolates that either produced large, clear plaques on *Escherichia coli* strain TD57 or yielded plaques that appeared to have a host range superficially resembling male-specific phages when first tested.

Portions of all 56 samples were plated on strains TD298 and TD299 for detection of male-specific phages. Pertinent methods have been presented in detail elsewhere (4) and will be presented briefly below.

Purification of phages and host range determination. Isolated plaques were stabbed with a sterile needle and shaken in 1 to 2 ml of TB containing a few drops of chloroform. Samples were plated to obtain isolated plaques. This step was successively repeated until pure cultures of phage were obtained. Host range was determined either by incorporating the test bacterium in the soft-agar layer onto which loopfuls of phage containing 10^4 to 10^5 PFU were placed, or phage suspensions were streaked across streaks of bacteria on NA plates.

Characterizaion of selected isolates of virulent phages. Phages T1, S13, and ϕX174 produce large, clear plaques on strain TD57. Hence, one or more plaques of such morphology were selected from indicator strain TD57 and were tested for their possible similarities to T1, S13, or ϕX174. Purified plaques were suspended in TB containing chloroform, and

TABLE 2. *Total PFU per gram (wet weight) of mammalian feces and the incidence of temperate coliphages*[a]

Cows		Pigs		Humans	
PFU × 10^2	Temperate[b] phage	PFU × 10^2	Temperate[b] phage	PFU × 10^2	Temperate[b] phage
2,240	P	790	P	1.7	P
1,940	P	32	P	28	A
820	A	360	A	11	P
260,000	A	0.4	A	4.6	P
60	A	160,000	P	2,710	P
16,900	A	16	P	0.25	P
7.2	A	20	P	15	P
4.5	A	207,000	A	3.1	P
470	A	2,080	A	9,200	A
0.1	A	14	A	33	P
		18.5	A	1.3	P
		61	P	0.4	P
		0.2	P	2.7	P
		0.1	P	0.5	P
		0.4	P	7,000	P
		2.7	P	1.0	A
		31,000	A	11	P
		162,000	P		
		14,000	A		

[a] PFU registering on strain TD57 (*E. coli*, strain C). Samples registering less than 10 PFU per g of feces are not listed in this table, but the numbers of such negative samples are presented in Table 3.
[b] Presence (P) and absence (A) of temperate phage plaques.

their host range was tested by spotting on TD230 and TD257. Isolates that lysed both of these strains were discarded. Isolates that lysed TD230 but not TD257 were presumed to be T1 related. Final identification as T1 was confirmed by studying their neutralization by anti-T1 serum as described before (7). Phage isolates that lysed TD257 but not TD230 were presumed to be related to either S13 or ϕX174, and this was confirmed by neutralization by anti-HK047 serum as described before (7). S13 and ϕX174 are not distinguishable by simple, qualitative serological tests. They can, however, be differentiated by strain TD262, which is sensitive to S13 (7) but is not lysed by ϕX174 (our unpublished data). Thus, host range tests carried out on this strain allowed identification of fecal phages as either S13 or ϕX174.

Virulent phages forming plaques on TD299 but not on TD298 can be either male-specific phages or host range M phages (HRM) (4). Male-specific phages grow on strain TD6 but not on strain TD244; HRM phages grow on both of these strains. The HRM isolates are of at least two different host range types: i.e., those related to HK219 and incapable of lysing strain TD248 and those related to HK221 and incapable of lysing strain TD252. All the HRM isolates tested by us (unpublished data) are either of HK219 host range or of HK221 host range. No antisera against phages HK219 and HK221 are available at present, and therefore "relatedness" of fecal isolates either to phage HK219 or to phage HK221 is defined by the sole criterion of host range.

Characterization of selected isolates of temperate phages. The first test involved a study of host range on bacterial strains TD57 and TD257. Strain TD257 is a *tonA* mutant of TD57 and was selected for resistance to coliphage T1 (see Table 1). *E. coli tonA* mutants are always resistant to phage HK022 (7) and, hence, newly isolated, temperate phages that failed to grow on TD257 were provisionally diagnosed as "related to HK022." Their final identity was confirmed by anti-HK022 serum neutralization tests (7). Phage isolates that grew on TD257 have not been further characterized.

Phage isolates related to HK022 were subjected to prophage immunity tests. Derivatives of bacterial strain TD9 singly lysogenic either for phage HK022 or for HK022-related phages of fecal origin were prepared as described before (2, 7). Lysogens were incorporated in the soft-agar layer of plaque assay plates and were spotted with approximately 10^6 cell-free PFU of HK022 and phages of fecal origin.

Cell-free PFU were prepared by exposing isolates of TD9 lysogenic for respective phage to ultraviolet (UV) irradiation. In general, log-phase cultures of 2 × 10^8/ml of CFU of density were exposed to 8 ergs of UV/mm^2 per s in the dark, and incubation was continued in the dark until cultures became clear. Lysates were made bacteria free by chloroform treatment and were clarified by centrifugation at 5,000 rpm for 30 min. Most lysates prepared in this manner assayed 10^{10} PFU/ml or more.

RESULTS

Total coliphage contents. Data on total coliphage contents of the feces of cows, pigs, and humans are given in Tables 2 and 3. The percentage of samples showing less than 10 PFU/g (negative samples) is 23, 9.5, and 23% for cows, pigs, and humans, respectively. Clearly, fecal

TABLE 3. *Frequency distribution of samples in classes defined by range of PFU per gram (wet weight)*

Range (PFU/g)	Animal and no. of samples		
	Cows	Pigs	Humans
<10 (negative samples)	3	2	5
10^1 to $<10^2$	1	4	3
10^2 to $<10^3$	2	1	6
10^3 to $<10^4$	1	6	5
10^4 to $<10^5$	2	2	0
10^5 to $<10^6$	2	1	3
10^6 to $<10^7$	1	2	0
10^7 or more	1	3	0

matter from each of the three mammals tested and especially that of porcine origin is an excellent source of coliphages. In quantitative terms, it does not seem possible to draw any generalizations. The PFU contents of different samples from a single species show widely divergent values, and this preliminary study does not offer any clues to the causes underlying such divergent PFU counts. Likewise, the data given in Tables 2 and 3 do not show any concrete differences in total PFU content when the three mammals are compared with one another. On the basis of the available data, it does appear that porcine samples generally tend to have higher PFU contents than samples obtained either from cows or humans. For example, 5 out of 21 porcine samples had more than 10^6 PFU/g, whereas only 2 out of 13 bovine samples and none out of 22 human samples registered high PFU counts. We did obtain data on total CFU per gram (wet weight) of feces for all the samples listed in Tables 2 and 3 (data not presented). The magnitudes of this parameter were almost as divergent as for the total PFU counts, and no evident correlations, either positive or negative, between these two parameters were perceptible. Whether the PFU contents will show any correlation with *E. coli* counts of different samples remains to be seen.

Incidence of specific phage types. A total of nine randomly selected samples were analyzed in greater detail. In eight samples identity of the phage occurring in largest numbers on one or the other indicator bacterial strain was established. The pertinent data are shown in Table 4.

Salmonella phages were detected in three samples out of five. Three plaques were selected from each sample, and after purification on *S. typhimurium* strain LT2 (TD28) they were spot tested on lawns of *E. coli* strains TD9 and TD57. None of the nine single plaque isolates lysed either of the two *E. coli* strains. Thus, within the limits of the above test, these phages may be regarded as *Salmonella* specific, unlike phage HK009 and its relatives which can grow equally well on both *S. typhimurium* and *E. coli* strains (7).

The most gratifying finding was the apparent confinement of some phages to the digestive tract of one or the other mammalian species. Whereas coliphage T1 was recovered from one sample each of cow and pig feces, phages related to the single-stranded deoxyribonucleic acid (ss-DNA) coliphages appeared to show some observable habitat preference. The well-characterized ss-DNA coliphages of the ϕX174 and S13 types are serologically closely related (14) but can be distinguished from one another by making use of *E. coli* K-12 strains made sensitive to phage S13 (7). The available data on the incidence of S13 and ϕX174 are presented in Table 4. Although the number of observations are not as numerous as might be desired, they nevertheless suggest that phages of the S13 type are more likely to be found in porcine fecal matter, whereas phages of the ϕX174 type may be encountered more frequently in fecal samples of bovine origin.

In a previous study of sewage samples, a nearly ubiquitous distribution of single-stranded RNA (ss-RNA)-containing coliphage was reported (4). It seemed of interest to search for such phages in mammalian feces. All 56 samples were appropriately analyzed; no male-specific phages, either ss-DNA- or ss-RNA-containing, were found. Phages of HRM (see Materials and Methods and reference 4) were recovered, but only from porcine samples. Whether these phages are actually absent from cow dung or their presence was eclipsed by

TABLE 4. *Incidence of specific types of phages in cow and pig dung*

Animal	Sample no.	*Salmonella* phages[a]	Identifiable virulent phage[b]
Cow	54	A	T1
	55	A	
	56	A	ϕX174
	57	A	ϕX174; HK243
Pig	58	A	T1; HK219
	59	P (2×10^3)	HK219
	60	P (8×10^2)	HK219
	61	P (6×10^2)	S13; HK221
	62	A	S13; HK221

[a] P denotes presence and A denotes absence of phages able to form plaques on strain LT2 of *S. typhimurium*. Figures in parentheses are the PFU per gram (wet weight) of feces.

[b] See Materials and Methods for a description of methods used for identification of different types of phages.

other phages present in larger numbers has not yet been established. The HRM-type phages obtained from five samples were further studied and showed either the host range of phage HK219 or phage HK221; no third type of host range was observed. A given sample yielded HRM phage either of HK219 or HK221 type but not of both types.

Phage HK243 produces the most unique plaque morphology ever observed by us. Its plaques are ordinarily hardly discernible but become clearly visible upon exposing the agar plates to chloroform vapors. This property is probably due to a very high degree of lysis inhibition, which correlates well with very high burst size of about 1,000 progeny PFU/infected cell (data not shown).

Temperate phages. This study was undertaken principally to develop methods for an efficient isolation of temperate coliphages from their natural habitats. Data in Table 2 show that this objective has been successfully realized. We conclude that, on the basis of the samples analyzed, cow dung is the least satisfactory source of temperate coliphages, and human feces are somewhat better material for the isolation of such phages than porcine fecal matter.

A serological examination of 23 temperate phage isolates was made. One of these has previously been shown to be related to coliphage P2 (2, 6). Out of the remaining 22 isolates, 20 were found to be inactivated by anti-HK022 serum and are thus serologically related to this latter phage (data not shown). The remaining two are serologically unrelated either to phage HK022 or to phage λ. From the HK022 homologues, 10 isolates were subjected to further analysis. All 10 are efficiently inducible by UV (Table 5).

Phage HK022 and 10 of the fecal isolates serologically related to HK022 were subjected to a prophage immunity test. Data in Table 6 show that these 11 phages (including HK022) fall into at least five prophage immunity classes. Prophage immunity group V may, in fact, represent more than one immunity group. This is particularly likely because HK73 in its prophage form exerts strong inhibitory effects on superinfecting heteroimmune phages HK74 and HK76. If the inability of any other member of group V to grow on HK73 lysogens can be shown to be due to exclusion as distinct from repressor-mediated immunity, a case for more than one immunity type is made. However, the appropriate experiments remain to be carried out. It is pertinent to mention here that, as far as immunity groups III to V are concerned, their members can be recovered from either animal species; e.g., out of the two members comprising group III, one (HK71) was obtained from pig dung, whereas the other (HK80) was recovered from human feces. The data in Table 6 allow the generalization that, of all the temperate coliphages occurring as free virions in nature and that could be detected by the indicator strains used, those serologically related to HK022 are by far the most abundant.

TABLE 5. *Ultraviolet light inducibility of temperate phages of fecal origin*[a]

Phage	Treatment and lysate titer[b]		
	Control	UV[c]	Control: UV
HK71	3.3×10^4	4.0×10^7	1.21×10^3
HK72	4.3×10^4	7.0×10^7	1.63×10^3
HK73	2.2×10^5	1.2×10^{10}	5.5×10^4
HK74	1.5×10^8	2.5×10^{11}	1.7×10^3
HK75	2.0×10^8	2.2×10^{11}	1.1×10^3
HK76	5.0×10^7	3.4×10^{10}	6.8×10^2
HK77	4.6×10^7	1.3×10^{11}	2.8×10^3
HK78	1.0×10^8	6.2×10^{10}	5.9×10^2
HK79	8.4×10^6	6.0×10^{10}	7.1×10^3
HK80	7.9×10^7	7.0×10^{10}	8.9×10^2

[a] UV inducibility of phages was studied in the respective lysogenic derivatives of *E. coli* strain TD9.

[b] Cell-free PFU per ml.

[c] Total UV dose of 200 ergs per mm^2.

DISCUSSION

It should be pointed out at the outset that our data were collected by using a limited number of bacterial indicator strains and any interpretation of such data must take cognizance of the special properties of the indicator stains used. For example, strain TD57 is a "rough" derivative of an unknown "smooth" parental strain. Thus, the PFU counts given in Table 2 do not provide any measure of the density of smooth-specific phages occurring in mammalian feces. *Salmonella* phage estimates given in Table 4 were likewise obtained by using a single strain, namely, TD28, which happens to be of the smooth colony type. Hence, the estimates of *Salmonella* phages given in Table 4 are again likely to be underestimates because our choice of the indicator has inevitably excluded the rough-specific phages from enumeration.

To the best of our knowledge, this is the first report on the phage contents of mammalian feces in quantitative terms. These observations, therefore, are their own point of reference, excluding the possibility of comparison and contrast with like data from other laboratories. We select the following aspects of our data for additional comments.

In the first instance we should like to allude

TABLE 6. *Immunity relationships of temperate phages serologically related to HK022*[a]

Cell-free phage	Lysis of bacterial strain TD9 lysogenic for:[b]										
	HK74	HK76	HK71	HK80	HK75	HK78	HK022	HK72	HK73	HK77	HK79
HK74	−	+	+	+	+	+	+	+	−	+	+
HK76	+	−	+	+	+	+	+	+	−	+	+
HK71	+	+	−	−	+	+	+	+	+	+	+
HK80	+	+	−	−	+	+	+	+	+	+	+
HK75	+	+	+	+	−	−	−	+	+	+	+
HK78	+	+	+	+	−	−	−	+	+	+	+
HK022	+	+	+	+	−	−	−	+	+	+	+
HK72	+	+	+	+	+	+	+	−	−	−	−
HK73	+	+	+	+	+	+	+	−	−	−	−
HK77	+	+	+	+	+	+	+	?[c]	−	−	?[c]
HK79	+	+	+	+	+?	−	+	−	−	−	−
Prophage immunity group:	I	II	III			IV			V		

[a] Phage HK022 was isolated earlier (7); isolates HK71 to HK80 were recovered during the course of this investigation. HK71 to HK75 were recovered from porcine feces, and HK76 to HK80 were recovered from human feces.

[b] +, Lysis; −, absence of lysis.

[c] Phage HK77 seems capable of inducing limited lysis of cells carrying prophage HK72 or HK79.

to the absence of any mean values of total PFU contents of the feces of any of the three species investigated. From a practical point of view, one consequence of this is that all samples must be approached in an empirical fashion. However, the general protocol that has been followed by us and has given acceptable results can be safely recommended.

The first goal of an ecological study is to enumerate the total organisms in a habitat, which is followed by relative frequencies of members belonging to different taxa. As far as bacteriophages are concerned, the second objective is going to be hard to achieve, not only due to its own intrinsic technical difficulties, but also because of a lack of well-defined and biologically meaningful taxonomic categories. To date, the only criterion that is biologically meaningful and yet applicable to population mixtures of diverse taxa is the criterion of serological relatedness. Its utility and efficiency improve substantially if applied in conjunction with host range tests. Our data at present are of too preliminary a nature and are rather scant to permit any generalizations. However, we have been able to show that such a dual approach is feasible to bacteriophage ecology, and, bearing in mind the small number of observations made, it appears that some virulent phage do show some habitat preferences. Whether these seeming habitat localizations are a direct reflection of the microbial flora of the animal or are in some manner influenced by the physiology of the mammal remains to be determined. Clearly, more data are needed to establish habitat preferences, if they exist, before inquiring into the underlying causative factors.

Complementary to the above is our observation on the absence of any apparent localization to the fecal matter of a single mammalian species by one serological class of phages, i.e., the temperate coliphages serologically related to HK022. Members of this group are widely distributed and are recoverable from the feces of humans as well as pigs. It is interesting that in a fairly wide search for temperate coliphages (results presented here and our unpublished data) we have not so far recovered any phage bearing serological relatedness to coliphage lambda, which is often cited as typical of temperate coliphages. Phages related to coliphage P2 are occasionally encountered as cell-free virions (e.g., HK239; references 2, 6; our unpublished data) and are the most frequent prophages in the naturally occurring strains of *E. coli* (1; our unpublished data). It is possible to understand the presence of P2-related phages as prophages because most members of the P2 family confer properties on the host cells that provide defense against the lytic effects of superinfection by a wide variety of unrelated phages (1, 2, 6). From our unpublished studies on phage HK022, we are unable to explain the near ubiquity of this coliphage on similar grounds. An untested, but possible, explanation could be that perhaps coliform bacteria inhabiting the mammalian digestive tract are more amenable to the propagation of this temperate coliphage than any other.

If a comparison of coliphage contents of mammalian feces and sewage (5, 8) is made, two

interesting contrasts appear: (i) the higher incidence of occurrence of temperate phages in pig and human feces than in the sewage samples; (ii) the complete absence of male cell-adsorbing coliphages from fecal samples analyzed by us in contrast to their very high densities in sewage (4). Two explanations can explain this difference. First, it is possible that we have simply not analyzed any samples from the mammal, either one of the three species studied by us or a different species, that offers the habitat for this phage. This seems to be unlikely because we have looked at over 50 fecal samples and have repeatedly observed recurrences of other types of phages. The alternative explanation is that male-specific phages are endemic to sewage, and that this milieu represents their natural habitat. Since phages of this host range (male cell adsorbing) can adsorb to cells harboring the infectious multiple drug resistance factor (R-factor [12]), it would be interesting to see if they can be recovered from the feces of animals undergoing antibiotic therapy and thus likely to be harboring the R-factor.

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LITERATURE CITED

1. **Bertani, L. E., and G. Bertani.** 1971. Genetics of P_2 and related phages. Adv. Genet. 16:199–237.
2. **Dhillon, E. K. S., and T. S. Dhillon.** 1973. *HK239*: a P_2 related temperate phage which excludes *rII* mutants of T_4. Virology 55:136–142.
3. **Dhillon, E. K. S., and T. S. Dhillon.** 1974. N-methyl-N'-nitro-N-nitrosoguanidine and hydroxylamine induced mutants of the rII region of phage T4. Mutat. Res. 22:223–233.
4. **Dhillon, E. K. S., and T. S. Dhillon.** 1974. Synthesis of indicator strains and density of ribonucleic acid-containing coliphages in sewage. Appl. Microbiol. 27:640–647.
5. **Dhillon, T. S., Y. S. Chan, S. M. Sun, and W. S. Chau.** 1970. Distribution of coliphages in Hong Kong sewage. Appl. Microbiol. 20:187–191.
6. **Dhillon, T. S., and E. K. S. Dhillon.** 1973. Mutants of phage *HK239* defective in excluding phages λ, *T4rII*, *P1* and *P2*. Mol. Gen. Genet. 127:249–254.
7. **Dhillon, T. S., and E. K. S. Dhillon.** 1972. Studies on bacteriophage distribution. II. Isolation and host range based classification of phages active on three species of *Enterobacteriaceae*. Jpn. J. Microbiol. 16:297–306.
8. **Dhillon, T. S., and E. K. S. Dhillon.** 1974. Studies on bacteriophage distribution. IV. Differences in efficiency of indicator strains and temporal variations in phage content of sewage. J. Chin. Univ. Hong Kong 2:435–447.
9. **Hayes, W.** 1973. The mechanism of genetic recombination in *E. coli*. Cold Spring Harbor Symp. Quant. Biol. 18:75–93.
10. **Hershey, A. D., and W. Dove.** 1971. *In* A. D. Hershey (ed.), Bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
11. **Jacob, F., and E. L. Wollman.** 1961. Sexuality and genetics of bacteria. Academic Press Inc., New York.
12. **Meynell, E., and N. Datta.** 1966. The relation of resistance transfer factors to the *F*-factor (sex factor) of *Escherichia coli* K12. Genet. Res. 7:134–140.
13. **Sasaki, I., and G. Bertani.** 1965. Growth abnormalities in *Hfr* derivatives of *Escherichia coli* strain *C*. J. Gen. Microbiol. 40:365–376.
14. **Sinsheimer, R. L.** 1968. Bacteriophage φX174 and related viruses. Prog. Nucleic Acid Res. Mol. Biol. 8: 115–169.
15. **Zinder, N. D., and J. Lederberg.** 1952. Genetic exchange in *Salmonella*. J. Bacteriol. 64:679–699.